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The significance of peroxisome function in chronological aging of *Saccharomyces cerevisiae*

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Published in:
Aging Cell

DOI:
[10.1111/acer.12113](https://doi.org/10.1111/acer.12113)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2013

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Lefevre, S. D., van Roermund, C. W., Wanders, R. J. A., Veenhuis, M., & van der Klei, I. J. (2013). The significance of peroxisome function in chronological aging of *Saccharomyces cerevisiae*. *Aging Cell*, 12(5), 784-793. <https://doi.org/10.1111/acer.12113>

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Supporting information

Supplementary tables

Table S1: Yeast strains used in this paper.

Strains	Description	Reference
WT	BY4742 MAT α <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	
Δ <i>pex3</i>	BY4742 Δ <i>pex3::KanMX4</i>	Euroscarf collection
Δ <i>pex5</i>	BY4742 Δ <i>pex5::KanMX4</i>	Euroscarf collection
Δ <i>pex6</i>	BY4742 Δ <i>pex6::KanMX4</i>	Euroscarf collection
Δ 3' <i>pex5</i>	BY4742 Δ 3' <i>pex5::KanMX4</i>	this study
Δ <i>pot1</i>	BY4742 Δ <i>pot1::KanMX4</i>	Euroscarf collection
Δ <i>tgl3</i>	BY4742 Δ <i>tgl3::KanMX4</i>	Euroscarf collection
Δ <i>pex3</i> Δ <i>pot1</i>	BY4742 Δ <i>pex3::KanMX4</i> Δ <i>pot1::NatMX4</i>	this study
Δ <i>pex7</i>	BY4742 Δ <i>pex7::KanMX4</i>	Euroscarf collection
WT GFP.SKL	BY4742 <i>pMET25-GFP.SKL/Zeo^R</i>	this study
Δ <i>atg1</i>	BY4741 Δ <i>atg1::KanMX4</i>	Euroscarf collection
Δ <i>atg1</i> Δ <i>pex3</i>	BY4741 Δ <i>pex3::KanMX4</i> Δ <i>atg1::NatMX4</i>	this study
Δ <i>pex5/PEX5</i>	BY4742 Δ <i>pex5::KanMX4</i> pRS316- <i>PEX5</i>	this study

Table S2: Primers used in this paper.

Primers	Sequence 5'-3'
Pex5UP	TATGCAAAGGTTTCATAAACGGAGAACCCTGATCGATGATAAAAGAAGA A-CAGCTGAAGCTTCGTACGC
Pex5DN	CTCTCTTCAAAGTCTCTATAACAGTATCATTGTACGTATTCAAGAGAGAT- GCATAGGCCACTAGTGGATCTG
Pex5.1	GGCGTCTTAATGAGTCACCT
Pex5.2	ATGCCTGGCTTCACTTCTTG
Pex5.5	ATCCGCTCAGAGTATCTTCG
Pex5.6	TCCATGTCTCTTCGCATAGG
Pex5.A	GCTTGCTGATTTTACCTGATGTATT
Pex5.B	GAGAGCTTTTCTCTCCCTGATAAAC
Pot1.1	CTACAGCTGCTAACGCTACACCGACCAA
Pot1.2	CTAGGATCCCTGTACTCAGAGCCACAAG
Pot1.3	CTAACTAGTGCCGCCGCCATCTT
Pot1.4	CTACCGCGGACGTTACCTCATATGGCTATCG
Pot1.5	GAGGCATGCACTTCGGATTA
Pot1.6	AATTCAACGCGTCTGTGAGG
Pot1.7	GACATCATCTGCCCAGATGC
Pot1.8	TGGAGGGGAAGAAGTGAGAG
GFPSKL-3	TATCCGCGGCGCGCAATTAACCCTCA
GFPSKL-4	TATGCGGCCGCGTAACGCCAGGGTTTT
MET25.1	GGCGTCAGATTTAGGTGGAT
ATG1up	TTCAAATCTCTTTTACAACACCAGACGAGAAATTAAGAAA- GACGGATCCCCGGGTTAATTA
ATG1down	GGTCATTTGTACTTAATAAGAAAACCATATTATGCATCAC- CGACACTGGATGGCGGCGTTA
Atg1.1	CTGGGGAAACAGAGAACAGT

Supplementary experimental procedures

Construction of $\Delta atg1\Delta pex3$ strains

ATG1 gene was deleted in $\Delta pex3$ cells by replacing the open reading frame with nourseothricin resistance gene {Goldstein, 1999 #32}. The *atg1::NatMX4* DNA fragment was amplified with ATG1up and ATG1down primers (Table S2) using pAG25 {Goldstein, 1999 #32} as template and transformed into $\Delta pex3$ cells. Correct insertion by homologous recombination was confirmed by colony PCR using Atg1.1 and Pot1.6 primers (Table S2).

Chronological aging experiment on peroxisome induction medium for *pex* mutants

Overnight cultures were grown in MM medium containing 0.5% glucose and required amino acids. Those cultures were then diluted twice at $OD_{600\text{ nm}} = 0.1$ in fresh MM containing 0.3% glucose and grown for 8 hours. After the last pre-cultivation step, cells were diluted in MM containing 0.25% ammonium sulfate, 0.05% yeast extract, 0.1% oleic acid and 0.05% Tween 80 and 0.1% glucose. Cultures were incubated at 30°C, 200 rpm. Survival was assayed by counting colony-forming units (CFUs) after 2 days of incubation at 30°C on YPD agar plates. 24 hours after the last dilution (D1) was considered as 100% of survival. The results shown are mean values and standard error of mean. Statistical analyses were determined using two-way ANOVA. A p value of less than 0.05 was considered as a significant difference.